REMARKS

The present invention relates in part to the use of affinity tags in recombinant fusion protein constructs. In particular, the claimed invention relates to affinity tags which comprise two or more modules capable of mediating binding to streptavidin.

Claims 1–34, 36, 37, 40–45 and 47-51 are pending in the application, with claims 16, 17, 32–34, 36, 37, 40–45, and 47-51 under examination. The balance of the claims have been withdrawn from examination by the Examiner in accordance with a restriction requirement.

Claims 16 and 37 are amended herein as requested by the Examiner. These amendments do not alter the scope of the claims or introduce new matter.

Reconsideration of the claimed invention is respectfully requested in view of the foregoing amendments and the remarks contained herein.

I. Informalities

Applicant is grateful for the Examiner's careful reading of the claims, and has amended claims 16 and 37 according to the Examiner's remarks regarding clarity and precision.

II. Rejection Under 35 U.S.C. §102(e)

The rejection of claims 16, 17, 32-34, 36-37, 42-45, and 47-51 under 35 U.S.C. §102(b) as allegedly being anticipated by Skerra and Schmidt, *Biomedical Engineering* 16: 79-86, 1999, is respectfully traversed.

A. The claimed invention relates to provision of a fusion protein comprising a streptavidin–binding peptide linked to a protein sequence of interest. As recited in claim 16, the streptavidin–binding peptide comprises a sequential arrangement of two modules with an amino acid sequence of –His–Pro–Baa– in which Baa is selected from the group consisting of

glutamine, asparagine and methionine. At least one of the modules comprises a sequence –His–Pro–Gln–Phe–.

As an initial matter, Applicant notes that Thomas Schmidt, the inventor named in the present application, is also named as an author of the cited Skerra and Schmidt article. This article is also summarized in paragraphs [0008] to [0011] of the present specification.

The cited article discuss two commercially available peptide sequences having binding properties towards streptavidin, invented by Drs. Skerra and Schmidt prior to the present application (*see, e.g.*, U.S. Patent 5,506,121, also discussed in paragraphs [0008] to [0011] of the present specification). These two sequences are:

- *Strep-tag*: Ala-Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (page 80, right column, top of page); and
- *Strep-tag II*: Asn-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (page 81, left column, first full paragraph).

While Skerra and Schmidt discuss the use of these previously known peptide affinity tags individually, nowhere does the article discuss any sequential arrangement of two such modules, as required by the present claims.

Anticipation requires that each and every limitation of the claims be present in the cited reference. In this case, no *prima facie* case of anticipation exists.

B. While no obviousness rejection has been raised with regard to Skerra and Schmidt, Applicant wishes to point out the substantial evidence of nonobviousness already of record in the present case. As discussed by Dr. Schmidt in paragraphs 8-11 of his previously submitted declaration, the present invention is drawn to improved fusion proteins and peptides able to selectively bind to substrates comprising streptavidin (which term means either or both naturally-occurring and mutein or optimized forms such as the commercially available STREP-TACTIN® peptide). The streptavidin-binding peptides of the present invention have the ability to bind strongly to their substrate under non-competitive conditions and yet may be easily displaced

under competitive conditions. This bimodal characteristic results from cooperative binding of a sequential arrangement in the peptide of at least two binding modules, wherein each module is able to independently bind either streptavidin or a streptavidin mutein. In this way, the multiple modules cooperate under non-competitive conditions in a synergistic manner to cause tight binding of the peptide to the substrate.

In the Schmidt declaration, comparative data is presented concerning binding to a streptavidin matrix of a fusion protein having a sequential arrangement of two peptide tags versus a single peptide tag. Under simple washing of the column with a buffer, the fusion peptide having a single tag begins to wash through the column, while the sequential tags bind more effectively. Under competitive elution conditions, however, each module of the sequential tags has to compete independently with binding of a free substrate binding molecule (or mimic thereof), *e.g.* biotin or biotin derivatives, thereby resulting in the effect that elution of the whole peptide that comprises the two sequentially binding modules is almost as fast as for a single module alone.

As described in the present specification (*e.g.*, in pages 3-8 and Example 3), the use of this sequential arrangement provides a surprising and substantial improvement in purification yield relative to the use of a single affinity tag under conservation of mild conditions for efficient elution by competitive displacement. This improvement in yield under preservation of mild conditions for the whole process has unquestionable practical benefit in the purification of recombinantly expressed proteins which often are labile and prone to denaturation under non-physiological conditions.

Furthermore, the surprising nature of this discovery is apparent from Szostak *et al.*, U.S. Patent No. 6,841,359, previously cited by the Examiner in the present case. In column 15, lines 63-66, the '359 patent reports that the presence of two HPQ (His–Pro–Gln) motifs does not confer high affinity binding. Likewise, column 10, lines 12-24 reports that binding to streptavidin is actually conferred by the entirety of a 38 residue peptide. In contrast, the present invention demonstrates that a much simpler sequential arrangement of binding motifs can provide sufficient binding affinity while maintaining the ability of the streptavidin binding

peptides to be readily eluted under competitive mild conditions, and thus can provide improved purification yields.

CONCLUSION

For the reasons set forth herein, Applicant respectfully submits that claims 16, 17, 32–34, 36, 37, 42–45, and 47-51 are in condition for allowance. Applicants respectfully request that the Examiner reconsider and withdraw the grounds for rejection set forth in the Office Action.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (619) 203-3186.

Respectfully submitted,

/Michael A. Whittaker/

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By: _____
Michael A. Whittaker
Reg. No. 46,230
Tel. No. (619) 203-3186
Fax No. (858) 683-0390

BIOTECHNOLOGY LAW GROUP
12707 High Bluff Drive
Suite 200
San Diego, CA 92103
Customer Number 35938